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Shifting sows: longitudinal changes in the periparturient faecal microbiota of primiparous and multiparous sows

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ABSTRACT

Knowledge of periparturient longitudinal changes in sow microbiota composition is necessary to fully understand her role in the development of the piglet microbiota, but also to improve gut health and performance of the sow in lactation. Primiparous sows face the challenge of partitioning nutrients to support maternal growth in addition to supporting foetal growth and the demands of lactation. Additional metabolic stress present during the periparturient period may induce changes in the microbiota profile between primiparous and multiparous sows. Using 16S rRNA gene sequencing, the study aimed to characterise the longitudinal changes in the periparturient microbiota and identify differences within the sow microbiota profile associated with parity. Faecal samples from primiparous ($n = 13$) and multiparous ($n = 16$) sows were collected at four different time points (day -6, -1, 3 and 8) in relation to farrowing (day 0). Microbiota richness was lowest on day 3 and -1 of the periparturient period ($P < 0.05$). Microbiota community composition, assessed by weighted and unweighted UniFrac distances, demonstrated longitudinal changes, with day 3 samples clustering away from all other sampling time points ($P < 0.05$). The relative abundance of several genera segregated gestation from lactation samples including *Roseburia*, *Prevotella 1*, *Prevotella 2*, *Christensenellaceae R-7 group*, *Ruminococcaceae UCG-002* and *Ruminococcaceae UCG-010* ($P < 0.01$). Furthermore, day 3 was characterised by a significant increase in the relative abundance of *Escherichia/Shigella*, *Fusobacterium* and *Bacteroides*, and a decrease in *Alloprevotella*, *Prevotellaceae UCG-003* and *Ruminococcus 1* ($P < 0.001$). Primiparous sows had overall lower periparturient microbiota diversity ($P < 0.01$) and there was a significant interaction between parity and sampling time point, with primiparous sows having lower microbiota richness on day -6 ($P < 0.001$). There was a significant interaction between sow parity and sampling time point on microbiota composition on day -6 and -1 (unweighted UniFrac distances; ≤ 0.01) and day 8 (weighted and unweighted UniFrac distances; $P < 0.05$). Whilst no significant interactions between sow parity and sampling day were observed for genera relative abundances, multiparous sows had a significantly higher relative abundance of *Bacteroidetes dgA-11 gut group* and *Prevotellaceae UCG-004* ($P < 0.01$). This study demonstrates that the sow microbiota undergoes longitudinal changes, which are collectively related to periparturient changes in the sow environment, diet and physiological changes to support foetal growth, delivery and the onset of lactation, but also sow parity.

Implications

Broadening our knowledge of longitudinal changes in the periparturient sow microbiota will help to increase understanding of the sow's role in the development of the piglet microbiota. We found that the sow microbiota is affected by time in relation to farrowing, which highlights the need to use time-matched samples to understand the relationship over time between sow and piglet microbiota. Differences between the microbiota of primiparous and multiparous sows may be linked to the severity of gestational/periparturient metabolic syndrome, warranting further research.

Introduction

The neonatal piglet gastrointestinal tract (**GIT**) is seeded with microbes present in the uterine tract during expulsion, on the sow skin/udder and faeces, and from the pen environment. The development of the microbiota during early life is now recognised as having a significant impact on host metabolism (Mulligan and Friedman, 2017) and health

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(Dou et al., 2017; Zhuang et al., 2019). Studies aimed at assessing the impact of the sow faecal microbiota on the development of the piglet microbiota have focused on a single sow microbiota sampling time point (the day before/of farrowing) for which all comparisons are made (Bian et al., 2016; Kubasova et al., 2017). The underlying rationale of these studies is that microbiota seeding of the piglet GIT is assumed to occur within the immediate postnatal period. However, the period of microbiota developmental plasticity which can result in long-term changes to mammalian health has not been well defined, with age-matched microbiota studies between piglets and sows lacking in the literature. It is possible that the relationship between the sow and piglet microbiota extends beyond the first day of parturition. Aviles-Rosa et al. (2019) highlighted the importance of neonatal coprophagy for piglet health, and thus the sow faecal microbiota and metabolites, during the first week of life. Denying piglets access to sow faeces for the first 7 days postpartum resulted in lower white blood cell counts, post-weaning average daily gain and feed intake.

As gestation progresses, increased stress is placed on the sow, with changes in physiology, endocrinology, metabolism and immunity all occurring at once to support foetal delivery and the onset of lactation (Baldwin and Stabenfeldt, 1975; Père and Etienne, 2007; Cheng et al., 2018; Farmer, 2018; Huang et al., 2019). Recent research has demonstrated that these changes are correlated with alterations in the microbiota from trimester one to three of gestation (Liu et al., 2019) and during the periparturient period (Cheng et al., 2018; Huang et al., 2019; Shao et al., 2020). Furthermore, during the periparturient period sows will transition from a gestation diet low in energy and high in fibre to an energy-dense lactation diet. Such changes in nutrient availability and digestibility are reported to alter the microbiota profile (Sappok et al., 2015). However, there is currently a lack of research investigating whether the transition from gestation to lactation affects the faecal microbiota of sows in a parity dependant manner.

Metabolic stressors occurring during late gestation and lactation are likely to be heightened in primiparous sows, which must also partition nutrients to support maternal growth and mammary gland development (Pluske et al., 1998). Psychological stress is known to induce microbiota dysbiosis in humans (Cryan and Dinan, 2012). Additional psychological stress during the perinatal period arises from housing of sows in farrowing crates, restricting movement and subsequently the ability for sows to express natural nest-building behaviours associated with parturition (Jarvis et al., 2002). In countries where gestation crates are no longer permitted (such as under EU Council Directive 2008/120), this psychological stress will be more profound in primiparous sows, who will not have previously been exposed to close confinement or the innate motivation to express farrowing behaviours (Jarvis et al., 2001). These factors may induce a different microbiota profile in primiparous and multiparous sows during the perinatal period. Understanding parity dependant changes in the microbiota could help to inform and tailor management strategies to promote better gut health and lactation performance.

The overall aim of the study was to understand the dynamic changes in the sow microbiota from 6 days prepartum to 8 days postpartum and determine whether these changes are affected by parity. We hypothesised that: (1) sow microbiota profile will undergo dynamic changes in response to the transition from gestation to lactation and (2) microbiota dysbiosis associated with this transition will have a greater impact on primiparous sows compared to multiparous sows.

Material and methods

Animal housing and management

A total of 29 Large White × Landrace sows (multiparous = pure Hermitage Seaborough Ltd., UK and primiparous = Hermitage Seaborough Ltd. × Rattlerow Farms Ltd., UK), from eight consecutive farrowing batches were used in this study. Sows were grouped based on parity as being primiparous ($n = 13$) or multiparous (second parity and above, $n = 16$, average parity = 2.63 (SD = 0.719)). For each sow, where possible a faecal sample was collected at four time points. Where a faecal sample could not be obtained a rectal swab was instead used to obtain a faecal microbiota sample. Rectal swabs and faecal samples are herein collectively referred to as faecal samples throughout the study. The four sampling time points were day 109 gestation (day −6), as this was the last day in straw-yard group housing for sows; 1 day before farrowing, as the neonatal microbiome is likely to be seeded with traces of this faecal material passed by sows; 3 days postpartum, when passing of faeces has resumed; 8 days postpartum, to collect faecal samples at the end of the periparturient period. These days are referred to as days in relation to farrowing (D0) for the duration of the paper (D -6, D -1, D3 and D8, respectively).

Gestating sows were managed in a three-week indoor batch farrowing system and housed in solid floored (concrete) barns in groups of five sows of similar size and parity. Each pen consisted of a kennelled lying area (depth 2.50 m × width 2.20 m) containing straw bedding, an outside dunging area (depth 2.15 m × width 2.20 m), in front of which there were five individual feeding crates (length 1.84 m × width 0.44 m per crate). A nipple drinker in the dunging area provided *ad libitum* water. During gestation, sows were fed a home-milled mash gestation diet based on barley and soybean meal (13.14 MJ digestible energy (**DE**)/kg, 13.82% crude protein (CP) and 0.62% standard ileal digestible (SID) lysine; Supplementary Table S1). They received approximately 2–2.50 kg/head per day at 0730 h daily throughout gestation.

Multiparous sows were moved from gestation group housing in solid floor barns with straw to a conventional part-slatted farrowing pen with a farrowing crate at approximately 109 days of gestation, with primiparous sows entering at 111 days. The later entry of primiparous sows into the farrowing house was standard practice on the commercial unit, reducing the amount of time primiparous sows spent in farrowing crates in an effort to reduce stress. Prior to entry, the farrowing pen was washed and disinfected (concentration = 0.03% PhenoPharm, East Riding Farm Services, UK) and allowed to dry for a minimum of 7 days. Farrowing crate dimensions were as follows: entire pen 1.80 m width × 2.42 m length, creep area 1.11 m length × 0.80 m width and sow crate 0.6 m width × 1.77 m length to the feed trough. All sows were wormed with Bimectin (5 ml primiparous and 8 ml multiparous intramuscularly (**IM**), Bimeda, Llangefni, UK) upon entry to the farrowing house and received a FarrowSure Gold vaccine the day before weaning (2 ml IM, Zoetis, Surrey, UK), which occurred at ~28 days postpartum. Following housing in the farrowing crates, sows received approximately 0.70 kg/head of the gestation diet feed twice daily at 0745 h and 1500 h until farrowing. The day after farrowing sows were transferred to a home-milled mash lactation diet (13.98 MJ DE/kg, 18.50% CP and 0.95% SID lysine; Supplementary Table S1) initially as a 2.0 kg/head per day allowance, which was increased to appetite by 0.5 kg/head per day until a 10 kg/head per day limit was reached. A change in diet was necessary to meet the increased nutrient requirements of the sow during lactation, and to study the change in the periparturient sow microbiota under conditions representative of commercial practice. Individual sow feed intakes, P2 measurements and liveweights were not recorded. Water was available *ad libitum* through a nipple drinker. Cross-fostering of piglets, to create uniform litters of piglets based on birthweight, occurred within the first 24 h postpartum. Litter size was set according to the number of functional teats. The number of piglets weaned per experimental sow was recorded at weaning. Piglets were managed according to Gaukroger et al. (2020). Veterinary records for both the sow and her litter were recorded throughout lactation. Any antibiotic treatment administered to the sows was recorded as penicillin treatment 'yes' or 'no' (yes $n = 5$ (two primiparous sows and three multiparous sows)). Sows were only treated with a three-day course of penicillin (10 ml IM, Pen & Strep, Norbrook, Newry, UK) if they presented thick creamy vaginal discharge, or symptoms of mastitis, metritis and agalactia. A description of sow farrowing performance and antibiotic

usage can be seen in Supplementary Table S2. No confounding differences in sow performance were observed that may cause a difference in the microbiota associated with sow parity, thus not included in the microbiota analysis.

16S rRNA gene sequencing

Bacterial DNA was extracted from 250 mg of faeces using the DNeasy PowerSoil HTP 96 kit (Qiagen, UK) following manufactures instructions and the centrifugation-based protocol for DNA binding and column-washing steps. The V4 region of the 16S rRNA gene was amplified by PCR. Library generation, quality control steps and sequencing procedure were conducted in accordance with the Kozich et al. (2013) standard operating procedure. Briefly, amplification was performed using high fidelity Accuprime Pfx SuperMix (Invitrogen, USA) with the following conditions: 95 °C 2 min, then 30 cycles of 95 °C for 20 s, 55 °C for 15 s and 72 °C for 5 min followed by a final step of 72 °C for 10 min. Amplicons were cleaned and normalised using the SequelPrep normalisation kit (Invitrogen, USA). Samples were pooled and quantified using the QuBit hsDNA kit (Invitrogen, USA) and fragment size was confirmed using the Agilent BioAnalyzer 2100 high sensitivity DNA kit (Agilent Technologies Inc., USA). The final library was loaded at 5pM with 10% PhiX and sequenced using an Illumina V2 500 cycle kit on the Illumina MiSeq (Illumina, USA). Sequencing was performed on the Illumina MiSeq using the 2×250 bp paired-end read protocol at NU-OMICS DNA sequencing facility. The bioinformatics methods reported by Stewart et al. (2018) were followed and are described in Supplementary Material S1. A total of 2 377 687 sequencing reads were obtained from an initial 104 sow samples run on the Illumina MiSeq. Sequences were rarefied to 3 500 reads per sample. After rarefaction 103 samples were retained, consisting of 22 phyla and 303 genera.

Statistical analysis

All statistical analyses were conducted in R version 3.6.2. Fixed effects considered in all models were day in relation to farrowing (Day), sow parity (Parity) and whether experimental sows received antibiotic treatment during lactation. Sow ID was specified as the random effect in all models as it formed the repeated measure in all analyses. Early analysis of alpha and beta diversity values revealed no significant difference in the microbiota of antibiotic treated vs non-antibiotic treated sows. Additionally, no bacterial genera were significantly different between antibiotic treated vs non-antibiotic sows. Based on the results of this analysis, antibiotic treated sows ($n = 5$) were retained in the dataset to increase sample size and antibiotic treatment was not considered as a fixed effect in subsequent statistical models. The number of sequencing reads for DNA extraction kit negatives and sequencing negative controls were inspected along with the microbiota community composition of DNA extraction kit negatives, sequencing negative controls and sequencing positive controls. Controls were deemed to not be representative of the sow microbiota and removed from further analysis (Supplementary Figure S1).

As previously described by Gaukroger et al. (2020), *post hoc* pairwise comparisons of significant fixed effects and interactions between significant fixed effects were determined using the 'emmeans' package (version 1.3.4), resulting *P*-values were Tukey adjusted for multiplicity as part of the 'emmeans' workflow. Adjusted *P*-values below 0.05 were considered statistically significant. All models were tested for validity, using two diagnostic plots. The first diagnostic plot consisted of a QQ plot of the standardised residuals, whilst the second was a scatterplot of the standardised residuals plotted against fitted values. All plots were generated by the 'ggplot2' package (version 3.1.1).

Observed operational taxonomic units (**OTUs**; richness) and Shannon diversity index (evenness) were calculated using the 'vegan' package (version 2.5). Generalised linear mixed effect models (**GLM**; 'lme4' package version 1.1.21) were used to determine any significant longitudinal changes in taxonomic richness and diversity associated with the fixed effects (Gaukroger et al., 2020). For the observed OTUs longitudinal GLM model the family function was specified as Poisson. As raw Shannon diversity index values were not normally distributed, they were subjected to a box cox normalisation using the *boxcox* function of the 'MASS' package (version 7.3–51.5) to calculate the best transformation, which was then applied to the Shannon values. A linear mixed effect model (**LME**) was then performed with normalised Shannon diversity index values with respect to the fixed effects using the 'nlme' package (version 3.1–145).

Beta-diversity distances (weighted and unweighted UniFrac) were generated using the 'rbiom' package (version 1.0.2.9002). The *Adonis* function of the 'vegan' package (version 1.0.2.9002) was used to assess, via a PERMANOVA with 999 Monte Carlo permutations, whether any of the fixed effects caused a significant longitudinal difference in either weighted or unweighted UniFrac distances. Weighted UniFrac distances take into account the relative abundances of taxa, whilst unweighted UniFrac distances are binary, giving equal weighting to rare and abundant taxa.

To determine the longitudinal changes in individual genera abundance associated with the periparturient period (**Day**), the effect of Parity and any interactions between Day and Parity, LME models were performed using the 'lmer' package (version 1.1–21). Prior to running LME models, genera abundances were filtered to only retain genera with an average relative abundance ≥0.001 (0.1%) and ≥10% prevalence; retained genera ($n = 77$) were then arcsine square-root transformed. *P*-values were false discovery rate (**FDR**) adjusted for multiplicity (Benjamini and Hochberg, 1995) and resulting *P*-values below 0.05 were considered significant. Example R scripts for each analysis can be seen in Supplementary Material S2.

Results

Longitudinal changes in sow microbiota during the periparturient period

Based on the results of the GLM, there was a significant effect of Day on the number of observed OTUs, with samples on D3 having a significantly lower number of observed OTUs (218, SE = 11.5) compared to all other sampling time points ($P < 0.05$; Fig. 1a). Furthermore, samples on D -1 (229, SE = 6.3) had significantly lower numbers of observed OTUs compared to samples taken at D -6 (239, SE = 9.8) and D8 (241, SE = 4.0). Day did not have a significant effect on Shannon diversity index values (Fig. 1b). Weighed UniFrac distances demonstrated that Day ($P = 0.001$; Fig. 1c) also had significant effects on sow microbiota composition. Similarly, analysis of unweighted UniFrac distances demonstrated a significant effect of Day (Supplementary Figure S2a). Taxonomic analysis at the phylum level showed the faecal microbiota was dominated by Bacteroidetes and Firmicutes across all timepoints, and to a lesser extent Spirochetes, Proteobacteria and Fusobacteria (on D3 only; Fig. 2). At the genus level, the faecal microbiota was dominated by *Treponema 2*, *Prevotellaceae NK3B31 group*, *Prevotella 1*, *Prevotella 9*, *Phascolarctobacterium, Lactobacillus, Rikenellaceae RC9 gut group, Ruminococcaceae UCG-005, Alloprevotella* and *Bacteroides* (Supplementary Figure S3). Based on the results of LME models, several significant longitudinal patterns in genera abundance were observed during the periparturient period. Patterns were determined by inspection of compact letter displays generated by Tukey's *post hoc* pairwise comparisons of adjusted mean values for each genus, with different letters denoting significant differences in mean relative abundance between sampling time points (Supplementary Table S3). The first pattern was a significant change in genera abundance between gestation and lactation days. This pattern was characterised by a significantly lower abundance of *Roseburia*, *Prevotella 1* and *Prevotella 2* in lactation, whilst the abundance of *Christensenellaceae R-7 group, Ruminococcaceae UCG-002* and *Ruminococcaceae UCG-010* were significantly higher. The second pattern was associated with significant changes in genera abundance occurring

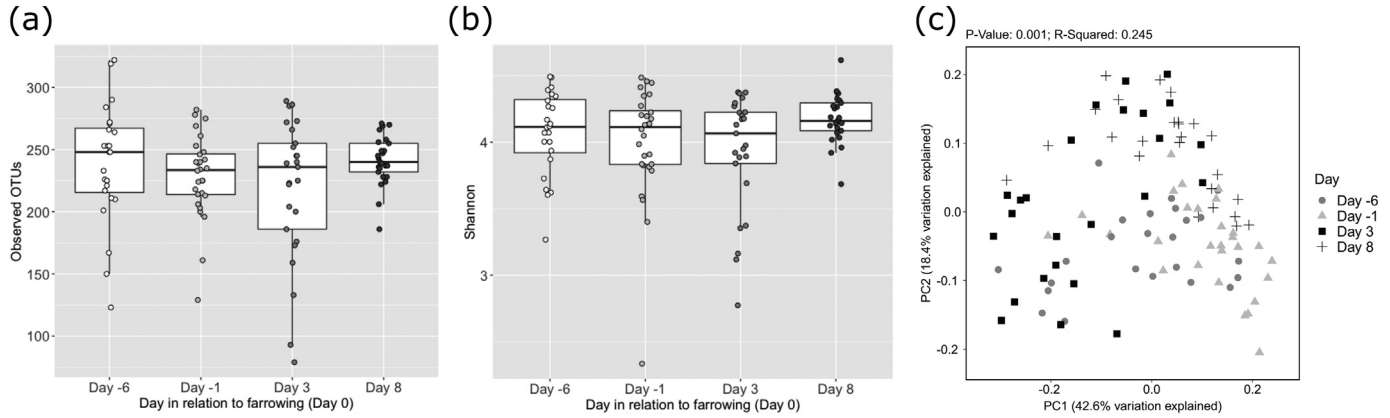

Fig. 1. Changes in (a) sow faecal microbiota richness (observed operational taxonomic units (OTUs)) and (b) eveness during the perinatal period in relation to farrowing (Day 0). (c) Principle coordinates analysis (PCoA) plot of the weighed UniFrac distance matrix, illustrating the significant changes in faecal microbiota community composition of sow faeces according to sample day in relation to farrowing (Day 0). Samples with similar microbiota community composition are positioned more closely to each other.

on D3. This second pattern included a significant increase in *Bacteroides*, *Escherichia/Shigella* and *Fusobacterium* abundances compared to all other time points. Conversely, several genera abundances were significantly decreased on D3 compared to all other sample days, namely *Alloprevotella*, *Prevotellaceae UCG-003* and *Ruminococcus 1* (Fig. 3).

Differences in sow microbiota related to Parity during the periparturient period and interactions between Parity and Day

There was a significant effect of Parity on alpha diversity measures. Multiparous sows had a higher number of observed OTUs (245 SE = 5.3 vs 215 SE = 6.0, $P < 0.001$) and Shannon diversity index (4.14, SE = 0.051 vs 3.92, SE = 0.046, $P < 0.01$) compared to primiparous sows (Fig. 4a and b). Furthermore, there was a significant interaction between Day and Parity; multiparous sows had a significantly higher number of observed OTUs on D -6 ($P < 0.001$; Fig. 4c), with the same trend observed on D3 ($P = 0.058$). No such interaction was observed for Shannon diversity index. There was a significant interaction between Day and Parity for weighted UniFrac distances on D8 ($P < 0.05$; Fig. 5), and on D-6, -1 and 8 for unweighted UniFrac distances (Supplementary Figure S2b). The results of the LME models also reported two genera to be significantly different in abundance across all time points between multiparous and primiparous sows, following FDR adjustment. Multiparous sows had a significantly ($P < 0.01$) higher abundance of *Bacteroidetes dgA-11 gut group* (1.67%, SE = 0.200 vs 0.58%, SE = 0.142%) and *Prevotellaceae UCG-004* (0.29%, SE = 0.040 vs 0.08%, SE = 0.020%). No significant interactions between Day and Parity were observed for relative genera abundances after FDR adjustment for multiplicity.

Discussion

There is currently a lack of research closely monitoring the microbiota changes associated with the periparturient period in sows and whether this is affected by sow parity. To the best of the author's knowledge, no studies have monitored microbiota changes associated with parity, microbiota changes occurring specifically within the last week of gestation, nor have they ascertained the immediate impact of farrowing on microbiota by comparing samples taken on the last day of gestation to samples collected once the resumption of postpartum

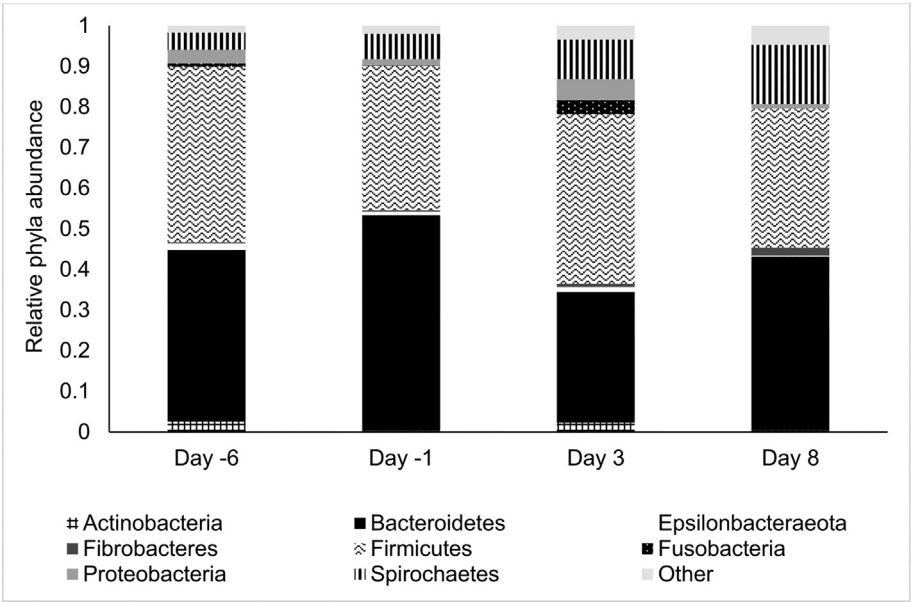

Fig. 2. The relative abundance of the top 9 phyla present in sow faeces according to sampling day relative to farrowing (Day 0).

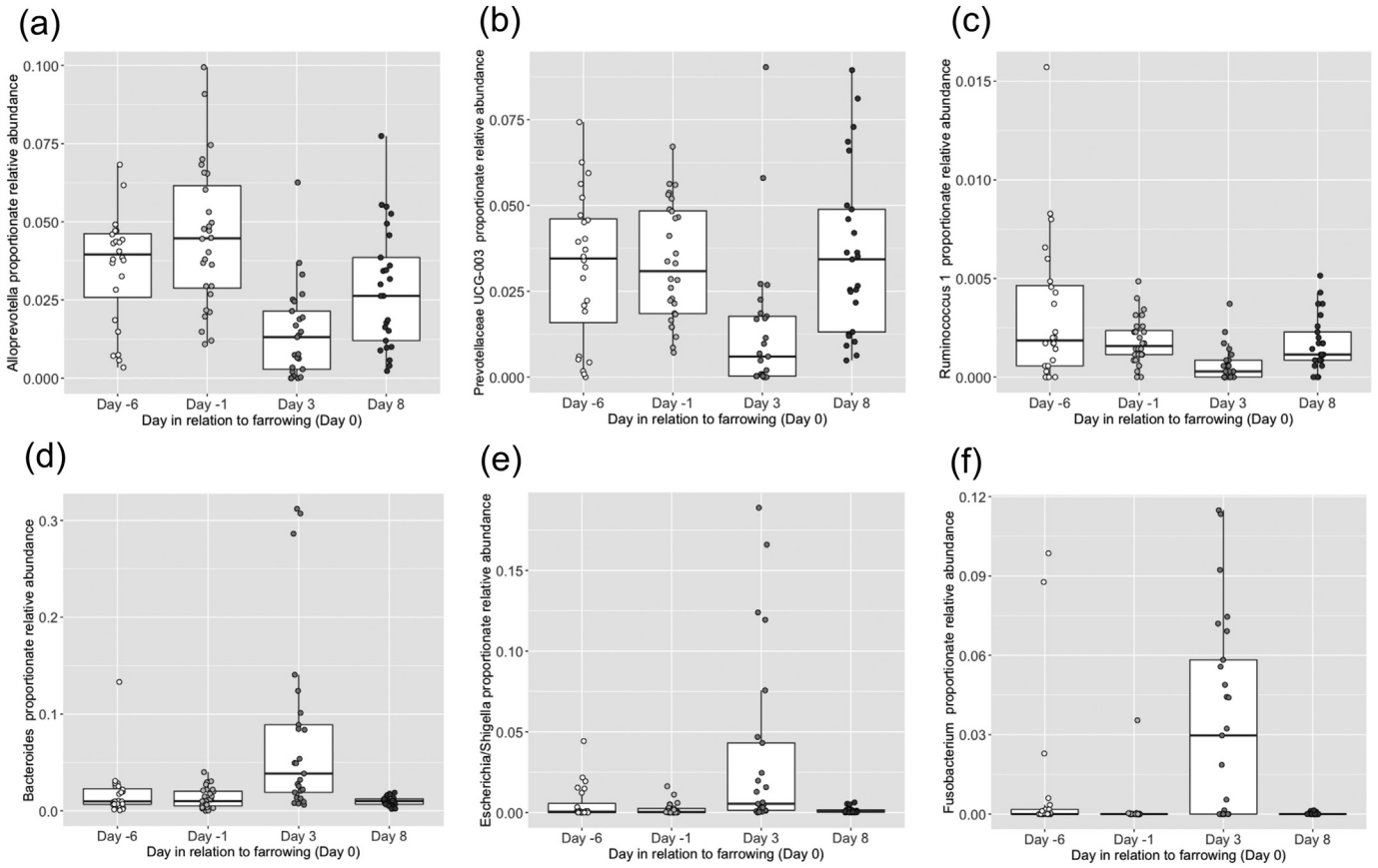

Fig. 3. Changes in the relative abundance of (a) *Alloprevotella*, (b) *Prevotellacese UCG-003*, (c) *Ruminococcus 1*, (d) *Bacteroides*, (e) *Escherichia/Shigella* and (f) *Fusobacterium* over time, with distinct changes occurring at 3 days postpartum compared to sow faecal samples collected on Day -6, -1 and 8 (in relation to farrowing (Day 0)).

bowel movements has occurred. We report longitudinal changes in the microbiota during the periparturient period and an effect of parity on the sow microbiota.

Longitudinal changes in the sow microbiota during the periparturient period

We hypothesised that the microbiota profile of sows will undergo dynamic changes in response to the transition from gestation to lactation. The number of observed OTUs was significantly lower on D -1 and D3 compared to other sampling time points, similar to Cheng et al. (2018). Liu et al. (2019) also observed a significant reduction in alpha diversity of sow faeces as gestation progressed, whilst Huang et al. (2019) observed samples collected during the periparturient period to have lower diversity than non-pregnant sow faeces. This study demonstrated that microbiota richness continues to decline within the last week of gestation. A reduction in microbiota diversity related to progressive gestation stage has been associated with symptoms of metabolic syndrome (Koren et al., 2012) which sows exhibit during late gestation and early lactation (Cheng et al., 2018; Huang et al., 2019). Metabolic syndrome in sows is characterised by reduced insulin sensitivity (Père et al., 2000; Père and Etienne, 2007) to support the increasing demands for foetal growth (Koren et al., 2012; Père and Etienne, 2019), accompanied by an elevation in levels of faecal pro-inflammatory cytokines, a reduction in faecal IL-10 and an increase in plasma zonulin concentrations (Cheng et al., 2018). The pro-inflammatory status during late gestation is thought to be beneficial for foetal and placental expulsion during parturition (Mor and Cardenas, 2010).

Beta diversity, according to both unweighted and weighted UniFrac distances, was significantly affected by Day, with samples collected on D3 clustering away from the other time points. Cheng et al. (2018) used Bray Curtis distances opposed to UniFrac but reported similar findings for samples collected on D3 of lactation compared to D109 of gestation and D14 of lactation. Furthermore, Liu et al. (2019) reported Landrace gestation samples to cluster separately from lactation samples based on Bray Curtis distances, but reported no difference between lactation samples. The results of the present study and Cheng et al. (2018) suggest the microbiota community composition to be distinct during early lactation in sows.

The predominant phyla associated with the periparturient period were Firmicutes, Bacteroidetes, Spirochaetes, Proteobacteria and Actinobacteria, and Fusobacteria on D3, as reported in previous studies (Cheng et al., 2018; Huang et al., 2019; Liu et al., 2019; Shao et al., 2020). Numerous genera segregated according to gestation or lactation abundances. Several butyrate-producing genera were significantly reduced in lactation, including *Subdoligranulum* and *Roseburia*. Butyrate is an important energy source for colonocytes/epithelial cells and therefore has an important role in maintaining barrier function. Cheng et al. (2018) reported a reduction in butyrate concentration in sow faeces during early lactation. As in this study, Huang et al. (2019) also reported a reduction in *Roseburia* and *Phascolarbacterium* during lactation. *Roseburia* is associated with total antioxidative capacity (Wang et al., 2018). Lactation samples were also characterised by an increase in *Ruminococcaceae UCG-002* and *Ruminococcaceae UCG-010*, as described by Shao et al. (2020) in hyperprolific sows. *Christensenellaceae R-7 group* was increased in lactation; this genus has been associated with increased serum triglyceride concentration in humans (Vojinovic et al., 2019) and thus may assist in assimilating nutrients to support lactation. Furthermore, Liu et al. (2015) reported Christensenellaceae family abundance to be positively associated with feed intake and energy expenditure, as in lactation.

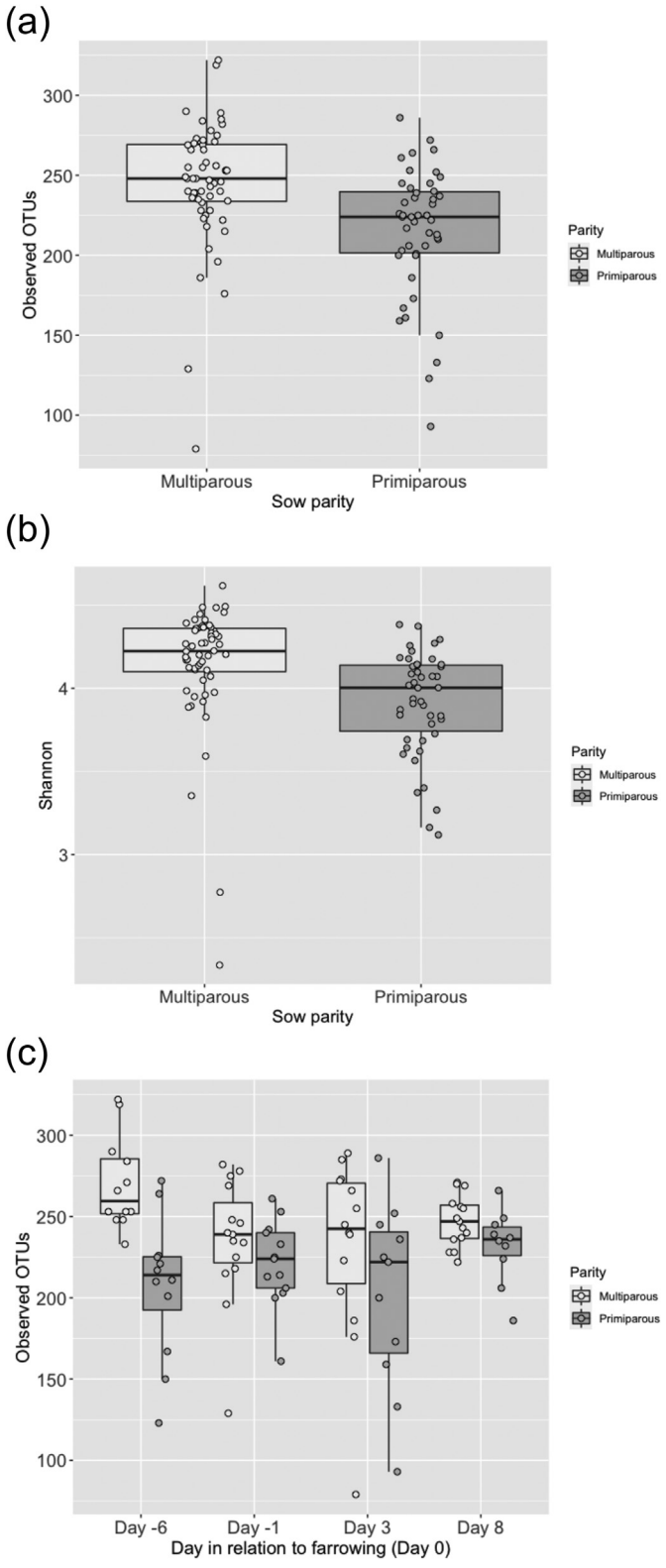

Fig. 4. Differences associated with parity in (a) richness (observed operational taxonomic units (OTUs)) and (b) evenness of diversity in sow faecal microbiota during the periparturient period and (c) the interaction between sampling day in relation to farrowing (Day 0) and sow parity with respect to microbiota richness.

Several genera abundances were also significantly affected on D3 of lactation in relation to the other sampling days. As reported by Shao et al. (2020), there was a significantly lower abundance of *Alloprevotella* and *Prevotellaceae UCG-003* on D3. In contrast, the relative abundance of *Bacteroides*, *Escherichia/Shigella* and *Fusobacterium* were significantly increased, as reported in the literature (Cheng et al., 2018; Shao et al., 2020). *Fusobacterium* abundance is negatively correlated with faecal IL-10 and positively associated with plasma zonulin in sows (Cheng et al., 2018). Liu et al. (2019) reported *Bacteroides* abundance to be negatively correlated with total short-chain fatty acid (**SCFA**) concentration of sow faeces. In this study, several SCFA producing bacteria had a significantly lower abundance on D3, including *Prevotella 9*, *Prevotellaceae UCG-003* and *Prevotellaceae NK3B31 group*. Not only does this reduce SCFA availability for sow metabolism to support the energy demands of lactation, but alterations in the concentration of SCFA could have increased GIT pH, creating an environment favourable for *Bacteroides* growth. Further research monitoring pH change of sow faeces during the periparturient period should be conducted to clarify this speculation. Sows often suffer from constipation around farrowing, Simreń et al. (2013) reported a significant increase in *Bacteroides* in patients with constipation predominant-irritable bowel syndrome. There was also a significant increase in *Escherichia/Shigella* abundance on D3, as previously reported (Cheng et al., 2018; Huang et al., 2019; Shao et al., 2020). Due to their genetic relatedness, 16S rRNA gene sequencing is unable to differential *Escherichia coli* from *Escherichia/Shigella* (Khot and Fisher, 2013). *Escherichia coli* are natural components of the sow microbiome, however, in a recent study it was demonstrated that giving mice an inflammatory stimulus caused certain strains of *E. coli* to increase the inflammatory response of the host, including IL-6 (Kittana et al., 2018). Cheng et al. (2018) observed that, on D3 of lactation, faecal IL-6 was increased, coinciding with an increase in *Escherichia/Shigella* abundance. It was also reported by Gaukroger et al. (2020) that *Escherichia/Shigella* and *Bacteroides* relative abundance was highest in piglets at 4 days of age during the first 8 weeks of life, correlating with the peak in the periparturient abundance of these genera.

Differences in the microbiota related to sow parity during the perinatal period

The study hypothesised that greater microbiota dysbiosis during the periparturient period would occur in primiparous compared to multiparous sows. To the best of the authors knowledge, no studies have compared the microbiota of primiparous to multiparous sows during the periparturient period. In this study, primiparous sows had a lower microbiota richness (number of observed OTUs) and evenness (Shannon diversity index) during the periparturient period compared to multiparous sows. There was a significant interaction between Day and Parity, with primiparous sows having lower microbiota richness on D -6, the last day of gestational housing in straw yards. The increased richness observed in multiparous sows may be associated with their possible higher intake of straw to alleviate any chronic hunger arising from gestational restriction feeding and larger maternal size/gut capacity. A reduction in insulin sensitivity has been associated with lower alpha diversity in sows (Cheng et al., 2018; Huang et al., 2019). As primiparous sows are required to partition more nutrients to support maternal growth compared to multiparous sows, in addition to nutrients to support foetal growth and lactation, it is possible that primiparous sows experience a further reduction in insulin sensitivity during the periparturient period. This may explain why alpha diversity is lower in primiparous sows during the periparturient period compared to multiparous sows. Whilst further research is required to determine this, George (1975) reported slower glucose clearance in younger sows. Future research should also record individual feed intakes, inflammatory and metabolic markers when comparing the microbiota of sows of different parities to determine how the severity of metabolic syndrome is affected by sow parity.

The microbiota community composition of primiparous and multiparous sows was significantly different on D8 according to weighed UniFrac distances; this may arise from the lower microbiota richness

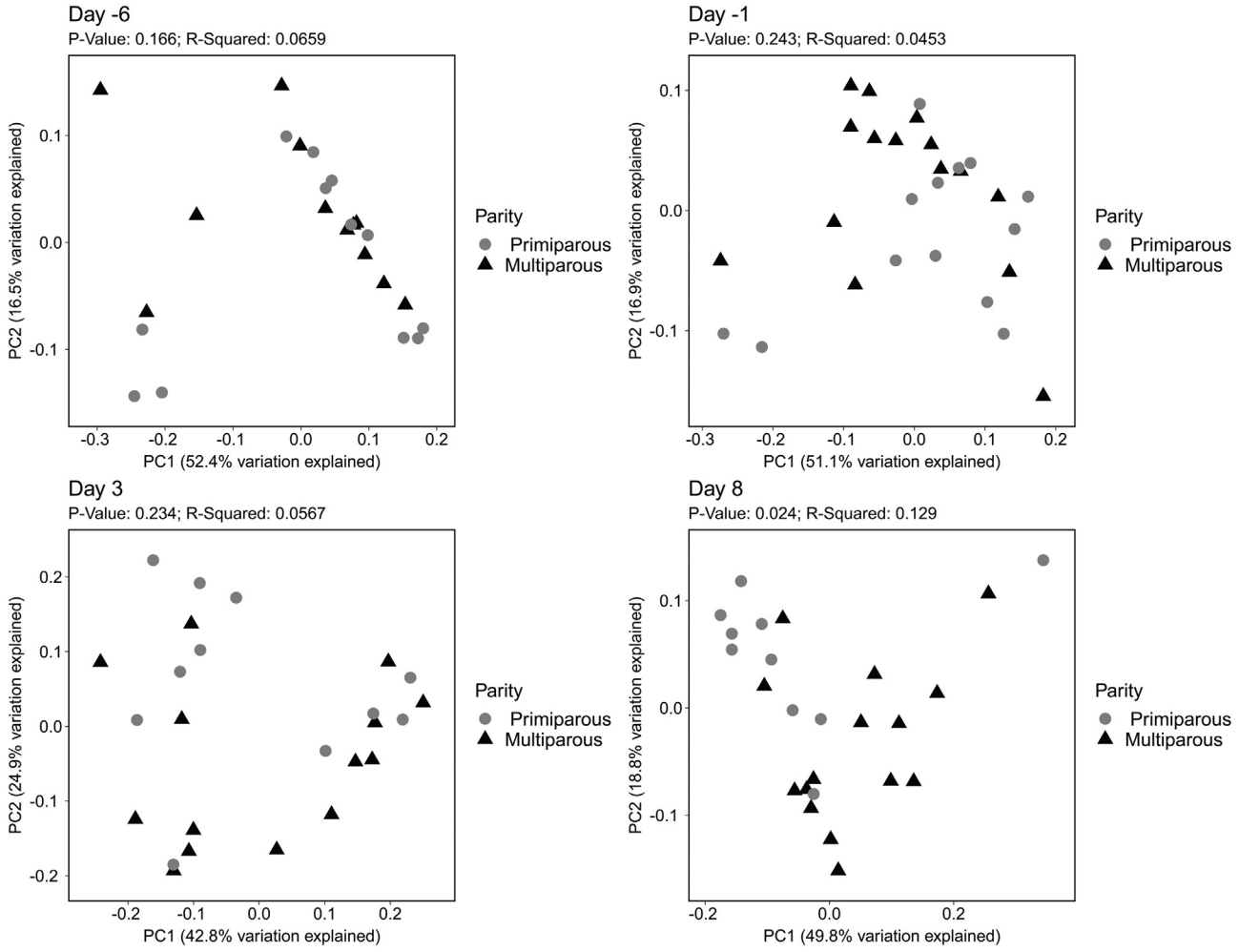

Fig. 5. Principle coordinates analysis (PCoA) plots of the weighed UniFrac distances, illustrating the significant changes in faecal microbiota community composition according to sow parity within each sampling day, in relation to farrowing (Day 0). Day is indicated by the number above each PCoA plot. Samples with similar microbiota composition are positioned more closely to each other. Significant differences in microbiota composition between parity groupings have a $P<0.05$.

and diversity present in primiparous sow faecal samples. Furthermore, microbiota community composition was significantly affected by parity on D -6, -1 and 8 for unweighted UniFrac distances, indicating that low abundance/rare taxa are the main driver of community divergence between parity groupings, especially on D -6 and -1. Across the periparturient period multiparous sows had a significantly higher abundance of *Bacteroidetes dgA-11 gut group* and *Prevotellaceae UCG-004*. Research monitoring faecal microbiota changes associated with parity in dairy cows also reported *Bacteroidetes dgA-11 gut group* to have a significantly higher abundance in multiparous compared to primiparous cows (Zhang et al., 2019a). Bacteria belonging to the Prevotellaceae family are commonly regarded as propionate producers. *Prevotellaceae UCG-004* has been positively correlated with carbohydrate metabolism and SCFA concentration in pig faeces (Zhang et al., 2019b), suggesting increased microbial fermentation in the hindgut of multiparous compared to primiparous sows.

In conclusion, we identified longitudinal changes in the periparturient sow microbiota profile. These findings corroborate previous literature, which deduced these microbiota changes to be associated with metabolic syndrome in sows. The significant microbiota changes occurring during the periparturient period highlight the need to utilise time-matched samples when determining the longitudinal effects of the sow on progeny microbiota development. Our study identified differences in the microbiota profile associated with sow parity, possibly suggesting that primiparous and multiparous sows are differentially affected by metabolic syndrome and perhaps its severity.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100135.

Ethics approval

Animal experimental procedures were conducted between January and July 2018 at the Pig Research Centre at Cockle Park Farm, Newcastle University (Ulgham, Morpeth, UK). All experimental procedures were conducted after approval from the Animal Welfare and Ethics Review Board (AWERB) of Newcastle University, Newcastle upon Tyne, UK (AWERB project ID no.555). Pig management and housing adhered to UK legislation and were in accordance with commercial standards specified by the Red Tractor assurance scheme.

Data and model availability statement

The raw sequencing reads and associated sample metadata analysed for this study were deposited in the NCBI Sequence read Archive (SRA)

under the BioProject accession number: PRJNA634147 https://www.ncbi.nlm.nih.gov/sra/PRJNA634147.

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Declaration of interest

Author CG received funding from Fera Science Ltd. and Newcastle University as part of an Institute for Agri-Food Research and Innovation postgraduate studentship. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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